Intraspecific structuring of *Polyommatus coridon* (Lycaenidae)

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Summary. The intraspecific differentiation of species is often a controversial matter. We analysed the population genetics of *Polyommatus coridon* (Poda, 1761), by means of allozyme electrophoresis over large regions of Europe, to obtain insight into patterns of intraspecific differentiation. We found significant population structuring (F_{ST}=0.060 ± 0.007 SD). A UPGMA analysis showed a division into two major genetic lineages that had a mean genetic distance (according to Nei 1978) of 0.041 (±0.010 SD). The analysed samples from Hungary, Slovakia, the Czech Republic and Brandenburg (north-eastern Germany) represented a monophyletic group, and those from Italy, France and Germany (excluding Brandenburg) another monophyletic group. The genetic differentiation within these two genetic lineages was rather weak. In general, genetic diversity within populations was high for all analysed parameters (number of alleles; observed and expected heterozygosity; percentage of polymorphic loci).

Zusammenfassung. Die intraspezifische Differenzierung von Arten wird häufig kontrovers diskutiert. Wir analysierten die Populationsgenetik von *Polyommatus coridon* (Poda, 1761) über weite Bereiche Europas mittels Allozymelektrophorese, um die intraspezifische Strukturierung dieser Art besser zu verstehen. Wir fanden eine signifikante Differenzierung der Populationen (F_{ST}=0.060 ± 0.007 SD). Eine UPGMA-Analyse ergab die Aufspaltung in zwei große genetische Linien, die eine durchschnittliche genetische Distanz (nach Nei 1978) von 0,041 (±0,010 SD) aufwiesen. Die analysierten Proben aus Ungarn, der Slowakei, Tschechien und Brandenburg (nordöstliches Deutschland) repräsentieren eine monophyletische Gruppe. Diejenigen aus Italien, Frankreich und Deutschland (ohne Brandenburg) stellen ein weiteres Monophylum dar. Die genetische Differenzierung innerhalb dieser beiden genetischen Linien war gering. Generell war die genetische Diversität innerhalb der Populationen für alle analysierten Parameter hoch: Anzahl an Allelen, beobachtete und erwartete Heterozygosität sowie der Prozentsatz polymorpher Loci.

Résumé. La différenciation intraspécifique des espèces est souvent une question controversée. Nous avons analysé la génétique des populations de *Polyommatus coridon* (Poda, 1761) au moyen de l'électrophorèse allozymique sur une large partie de l'Europe, afin d'obtenir une compréhension des modes de differenciation intraspécifique. Nous avons trouvé une structuration de populations significative (F_{ST}=0.060 ± 0.007 SD). Une analyse par agglomération hiérarchique (UPGMA) a démontré une division en deux lignées génétiques majeures ayant une distance génétique moyenne (d'après Nei 1978) de 0.041 (±0.010 SD). Les échantillons analysés d'Hongrie, de Slovaquie, de République Tchèque et du Brandenbourg (Allemagne du nord-est) représentent un ensemble monophylétique, et ceux d'Italie, de France et d'Allemagne (à l'exclusion du Brandenbourg) une autre ensemble monophylétique. La différenciation génétique à l'intérieur de ces deux lignées génétiques était assez faible. En général, la diversité génétique à l'intérieur des populations était élevée pour tous les paramètres analysés (nombre d'allèles; hétérozygosité prévue et observée, pourcentage de locus polymorphiques).

Key words. intraspecific differentiation, allozyme electrophoresis, *Polyommatus coridon*, *borussia*, *Lysandra*, *Meleageria*.

Introduction

During the 19th and the first half of the 20th century, taxonomy was one of the most prominent fields in biology. Numerous genera, subspecies, forms and aberrations of butterflies were described at this time (e.g. Seitz 1909, 1932). Recent trends have been towards reducing the number of taxa, and trying to obtain a more phylogenetically rigorous system (e.g. Nässig 1995). At a subspecific level, many formerly proposed taxa are no longer considered to be valid (e.g. Leraut 1997).

For *Polyommatus coridon* (Poda, 1761), the taxonomic situation is comparable to many other butterflies. In the past, many subspecies were described on the basis of minor morphological differences, with different subspecies often having peculiar distribution patterns (e.g. Seitz 1909, 1932). In a morphology-based revision of the *Lysandra*-group, Schurian (1989) reduced the number of subspecies to six: the nominate form in western and south-eastern Europe, *P. coridon borussia* (Dadd, 1908) in eastern Europe, *P. coridon asturiensis* (de Sagarra, 1924) restricted to northern Spain, *P. coridon caelestissimus* (Verity, 1921) endemic in central Spain, *P. coridon apennina* (Zeller, 1847) from central Italy and *P. coridon nufrellensis* (Schurian, 1977) endemic to Corsica. Not mentioned by Schurian (1989) were additional populations in Sardinia, which have been described as subspecies *P. coridon gennargenti* (Leigheb, 1987).

The aims of this work were to obtain data on the genetic structure of *P. coridon* and to re-analyse the intraspecific differentiation. Therefore, we sampled butterflies in an area that extended from the central Pyrenees to north-eastern Hungary and from central Italy to the Baltic Sea (see Fig. 1) and performed allozyme electrophoresis.

Ecology and distribution pattern of *P. coridon*

Polyommatus coridon is a characteristic species of barren grasslands on base-rich soils (Weidemann 1986; Ebert & Rennwald 1991; Settele et al. 1999). Its myrmecophilous larvae mainly feed on *Hippocrepis comosa* (de Bast 1987; Ebert & Rennwald 1991). Larvae of eastern populations feed on *Coronilla varia* (Schurian 1989; Settele *et al.* 1999).

The distribution range extends from the north of the Iberian Peninsula (Fernández-Rubio 1991) and the south-east of England (Emmet & Heath 1990) over major parts of temperate Europe (Tolman & Lewington 1998). *Polyommatus coridon* is nearly absent in the Netherlands (Wynhoff *et al.* 1992) and northern Germany (Bink 1992). In Poland, it can be found as far north as the Baltic Sea (Buszko 1997). In the Balkans, it reaches northern and central Greece (Pamperis 1997). No populations are known from Scandinavia (Henriksen & Kreutzer 1982). In the east, *P. coridon* can be found as far as the steppes north of the Lake Caspi (Lukhtanov & Lukhtanov 1994). Only one individual has been recorded from Turkey (Hesselbarth *et al.* 1995). Some authors classify Spanish populations as distinct species (e. g. Manley & Allcard 1970; Mensi *et al.* 1988).

Materials and Methods

Collection and allozyme electrophoresis. We collected butterflies at 36 localities (Fig. 1) and immediately stored them in liquid nitrogen until analysis. Half of the abdomen of each individual was homogenised in Pgm-buffer (Harris & Hopkinson 1976) by ultrasound and centrifuged at 17,000 g for 5 min. For the analysis, we applied cellulose acetate electrophoresis (Hebert & Beaton 1993). A total of 17 enzyme systems representing 20 loci were analysed. Four buffer systems were used. The electrophoresis conditions for the different enzymes are given in table 1.
The discrimination between some alleles of Ldh was not always possible. Therefore, the results for this enzyme were excluded for all calculations of genetic distances and all further calculations based on these values.

Data analysis

All loci showed banding patterns consistent with known quaternary structures and with autosomal inheritance (Richardson *et al.* 1986). The slowest migrating allele was labelled "1", the second "2" and so on.
Allele frequencies, F-statistics (Weir & Cockerham 1984), Nei's standard genetic distances (Nei 1978) and RxC χ^2-tests (Sokal & Rohlf 1995) were calculated with the

Tab. 1: Conditions of electrophoresis for the different enzymes tested. – TB: Tris-borate pH 8.9 (adjusted from TB pH 7.0 (Shaw & Prasad 1970)), TC: Tris-citrate pH 8.2 (Richardson *et al.* 1986), TG: Tris-glycine pH 8.5 (Hebert & Beaton 1993), TM: Tris-maleic acid pH 7.0 (adjusted from TM pH 7.8 (Richardson *et al.* 1986)). All buffers were run at 200 V. – * moves towards cathode; ** cold buffer necessary

enzyme	EC-Nr.	number of loci	buffer	Homogenate applications	running time (min)
6-Pgdh	1.1.1.44	1	TC	2	40
G-6-Pdh	1.1.1.49	1	TC	2	40
G-3-Pdh	1.2.1.12	1	TC	2	30
Gpd	1.1.1.8	1	TM	3	45
Hbdh*	1.1.1.30	1	TG	3	30
Idh	1.1.1.42	2	TC	2	40
Ldh	1.1.1.27	1	TB	5	40
Mdh	1.1.1.37	2	TC	2	40
Me	1.1.1.40	1	TB	2	30
Fum	4.2.1.2	1	TC	3	45
Aat	2.6.1.1	2	TG	3	45
Acon	4.2.1.3	1	TM	4	50
Pep D $_{(Phe\text{-}Pro)}$	3.4.11/13	1	TM	4	30
Apk	2.7.3.3	1	TG	1	30
Ak	2.7.1.40	1	TC	3	45
Pgi	5.3.1.9	1	TG	1	40
Pgm**	5.4.2.2	1	TG	1	40

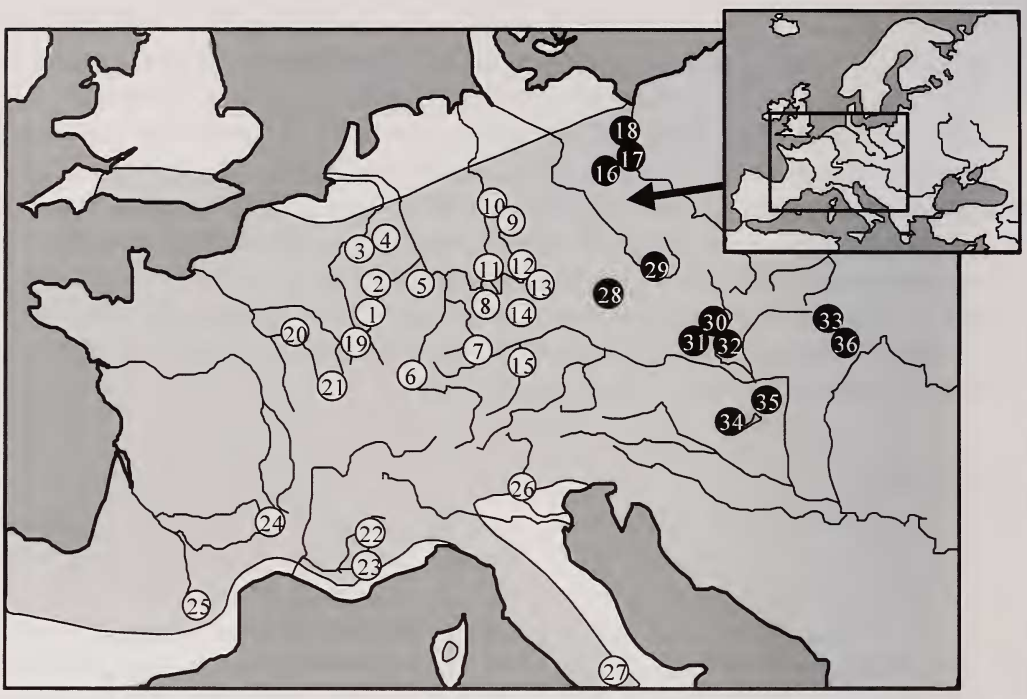

Fig. 1. Sampling localities of *Polyommatus coridon*. The species' distribution (Tolman & Lewington 1998, modified) is marked by a lighter gray area. Population numbers refer to those in Fig. 2. White circles with black population number: populations of the western genetic lineage; black circle with white number: populations of the eastern lineage.

program G-Stat (Siegismund 1993). Hardy-Weinberg equilibrium (Louis & Dempster 1987), genetic linkage disequilibrium (Weir 1991) and the exact tests (Raymond & Rousset 1995a) were performed with the package GENEPOP (Raymond & Rousset 1995b). Neighbor-joining (Saitou & Nei 1987) and UPGMA diagrams based on Nei's (1978) genetic distances were calculated with the package PHYLIP (Felsenstein 1993). Differences between means were tested with two-tailed t-tests, using STATISTICA (Stat Soft inc. 1993). Sequential Bonferroni corrections were performed as described in Rice (1989).

Results

Polymorphisms were observed for all analysed loci. The minimum number of alleles per locus over all 36 populations was three for Acon; the maximum was 16 for Pgi. The mean number of alleles per locus over all populations was 7.7 (±3.2 SD). Allele frequencies are available on request from the authors.

Genetic variability within each population was high: the average number of alleles detected per locus per population was 2.68 (±0.33 SD), ranging from 1.9 to 3.5. The mean percentage of polymorphic loci was 74.2% (±9.2 SD) (minimum 55%; maximum 95%). Restricted to loci with the most common allele not exceeding 95%, the mean

Tab. 2. Average number of alleles per locus (alleles), expected (He) and observed (Ho) percentage of heterozygosity, percentage of polymorphic loci (P tot) and on 95%-level (P95) for all analysed samples of *P. coridon*; ind: number of individuals examined. The averages are given with standard deviations. – Names of sample sites are abbreviated. Population numbers refer to Fig. 2.

	1 Perl	2 Dock	3 Wein	4 Niede	5 Münst	6 Griesh	7 Dapfe	8 Impfi	9 Craula	10 Hessel
alleles	2.40	2.50	2.50	2.75	2.60	2.70	2.80	2.75	2.75	2.65
He	19.2	19.4	19.0	19.4	21.1	18.7	21.6	18.7	21.1	19.3
Ho	18.5	19.7	17.1	19.8	21.5	19.5	21.5	17.6	20.3	17.7
P tot	75.0	75.0	60.0	80.0	70.0	85.0	90.0	80.0	75.0	65.0
P95	60.0	50.0	45.0	55.0	45.0	45.0	50.0	50.0	50.0	50.0
ind.	39	40	44	45	37	49	39	45	43	50

	11 Neust	12 Tiefen	13 Staad	14 Zimm	15 Tölz	16 Pätz	17 Libbe	18 Gartz	19 Chauv	20 Nogen
alleles	2.65	2.60	2.60	2.45	2.70	2.00	2.35	1.90	2.65	3.25
He	18.7	20.3	18.2	19.3	20.1	19.5	19.8	17.0	21.3	20.1
Ho	17.8	19.0	18.1	19.0	19.8	19.9	18.4	16.1	20.2	19.3
P tot	75.0	75.0	75.0	75.0	60.0	55.0	80.0	55.0	75.0	85.0
P95	50.0	45.0	45.0	50.0	45.0	55.0	65.0	50.0	55.0	60.0
ind.	40	41	50	40	50	48	40	33	42	50

	21 Velars	22 Barce	23 Palud	24 Langu	25 Carol	26 Baldo	27 Sasso	28 Černín	29 Milov	30 Lažan
alleles	3.30	3.50	3.05	3.25	2.60	2.35	2.95	2.40	2.55	2.55
He	20.1	23.8	20.1	23.1	21.1	19.0	22.7	19.3	20.2	19.7
Ho	19.5	23.1	18.0	23.6	22.1	18.2	22.7	17.4	19.5	19.0
P tot	80.0	95.0	85.0	80.0	75.0	60.0	75.0	70.0	70.0	80.0
P95	45.0	60.0	55.0	60.0	60.0	45.0	50.0	55.0	65.0	50.0
ind.	53	40	40	40	36	56	39	45	50	40

	31 Klent	32 Hradiš	33 Spišsk	34 Rezi	35 Csákv	36 Arany	average	min.	max.
alleles	2.75	2.80	2.40	2.90	2.90	2.80	2.68 ± 0.33	1.9	3.5
He	21.9	18.4	19.1	20.7	18.5	20.3	20.0 ± 1.5	17.0	23.8
Ho	18.9	19.6	18.5	20.6	18.8	19.7	19.4 ± 1.7	16.1	23.6
P tot	75.0	80.0	65.0	75.0	75.0	65.0	74.2 ± 9.2	55.0	95.0
P95	65.0	60.0	50.0	60.0	55.0	60.0	53.2 ± 6.5	45.0	65.0
ind.	47	45	50	40	40	40	43.5 ± 5.3	33	56

percentage of polymorphic loci was 53.2% (±6.5 SD) (minimum 45%; maximum 65%). The high percentage of polymorphic loci coincided with high observed heterozygosities (average 19.4% (±1.7 SD), ranging from 16.1% to 23.6%). Based on Hardy-Weinberg equilibrium, the expected values were even higher (mean 20.0%; minimum 17.0%; maximum 23.8%). All data are given in detail in table 2.

Linkage disequilibrium between loci was not detected after sequential Bonferroni correction.

Over all populations and loci, no significant deviation from Hardy-Weinberg equilibrium was detected ($p>0.99$). No single sample deviated from Hardy-Weinberg equilibrium at the 5% level except for Bad Tölz ($p=0.041$), which was not significant after Bonferroni correction. No single locus deviated except 6-Pgdh ($p=0.026$) and Hbdh ($p=0.008$), which were not significant after Bonferroni correction. Therefore, further analyses were performed using the standard statistics of population genetics.

A highly significant differentiation between the studied populations was revealed by means of an exact test ($p<0.0001$). Based on all analysed loci, the genetic distances (Nei 1978) between samples ranged from 0.014 (Zimmern and Bad Tölz, Bavaria; Nogent sur Seine and Velars, north-eastern France) to 0.069 (Carol, Pyrenees and Černín, western Bohemia). A neighbor-joining and a UPGMA phenogram (Fig. 2), which were calculated based on these genetic distances, showed a clear division into a western and an eastern group. All samples from Brandenburg (NE Germany), the Czech Republic, Slovakia and Hungary clustered in the eastern group. The western group included all samples from France, Italy and Germany (excluding Brandenburg). Each group exhibited several alleles not found in the other group. Some of these alleles were geographically widely distributed within the respective group (e.g. Mdh II allele 7, Gpi allele 10 and Aat II allele 9 in the western group; Idh I allele 4 and Idh II allele 8 in the eastern group). The mean genetic distance (Nei 1978) between these two groups was 0.041 (±0.010 SD). The mean genetic distances within the western and the eastern groups were 0.020 (±0.004 SD) and 0.022 (±0.003 SD), respectively. They were significantly lower than the distances estimated between these two groups (both t-tests: $p<0.0001$). F_{ST} calculated for all populations was significantly different from zero (0.060±0.007 SD). Within each group F_{ST} was much lower but still significant (west 0.023±0.003 SD; east 0.029±0.006 SD).

Discussion

Intraspecific differentiation. – Our allozyme studies showed that *P. coridon* splits into two well distinguished genetic lineages in the study area. This is consistent with de Lesse's (1969) two chromosome groups: a western group with 87 or 88 chromosomes occurring in Italy, southern France and northern Spain, and an eastern group with 90 to 92 chromosomes in the Balkans. Phenotypic differentiation, based on adult wing patterns, between eastern and western populations appears to correspond to the allozyme differentiation (Schmitt in prep). The distribution of the two distinguished genetic lineages is shown in Fig. 1.

Each of these two genetic lineages of *P. coridon* seems to be monophyletic. This is supported by several group specific alleles within each lineage.

Comparing with other population genetic studies of butterflies, the genetic distance (Nei 1978) of 0.041 between the two large lineages of *P. coridon* indicates at least subspecific differentiation. Similar values were found in other butterfly taxa for which subspecies are accepted (e.g. Porter & Geiger 1988; Britten *et al.* 1995; Descimon 1995). The observed genetic distances and F_{ST} values within the two large genetic lineages were typical for butterflies without subspecies (e.g. Vawter 1977; Eanes & Koehn 1978;

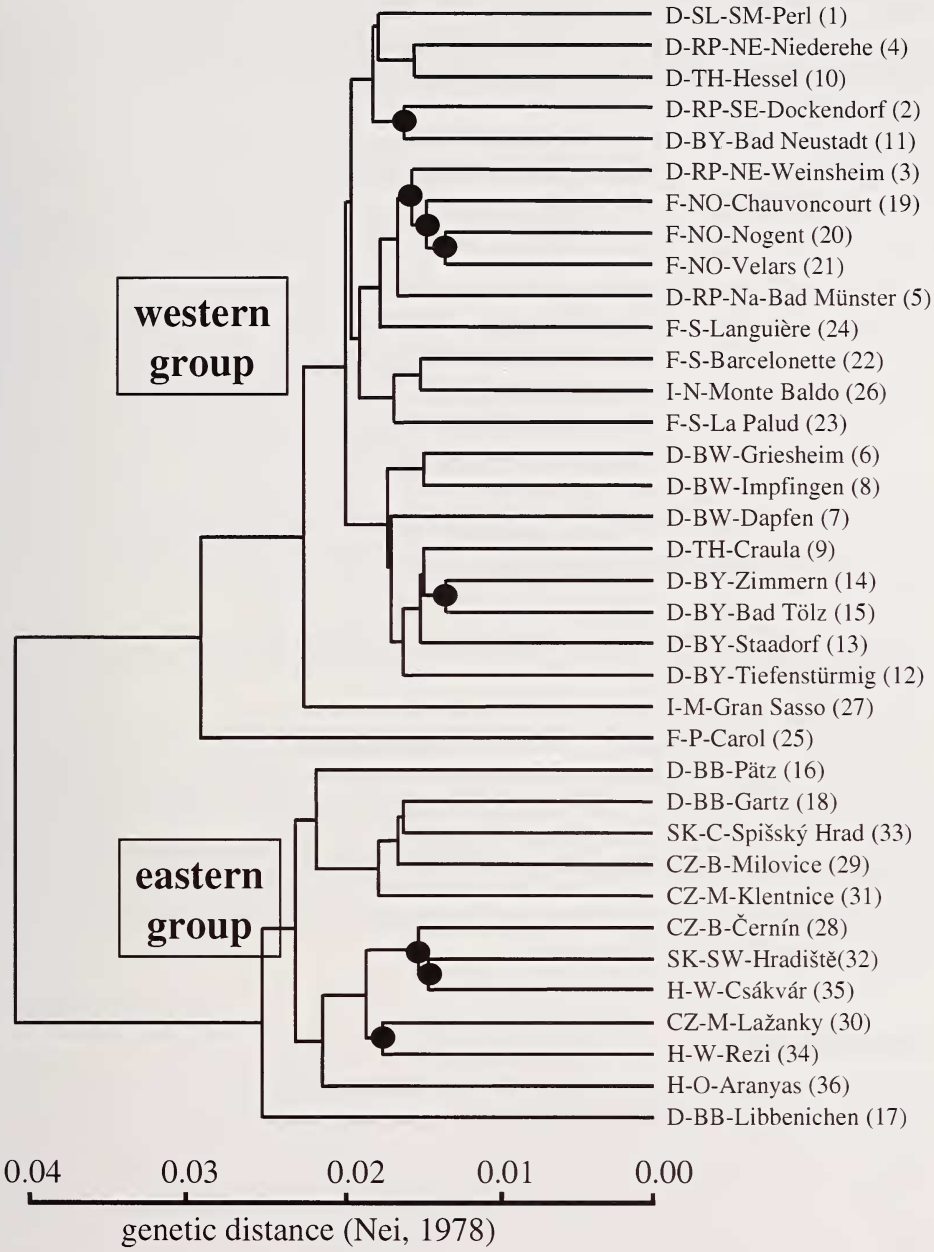

Fig. 2. UPGMA phenogram of all analysed samples of *P. coridon* based on genetic distances (Nei 1978). All nodes are inhomogeneous on the 5% confidence level before Bonferroni correction (RxC χ^2 test). Those which were not significant after Bonferroni correction are marked with filled circles. Abbreviations: *First part:* country; *Second part:* B: Bohemia, BB: Brandenburg, BW: Baden-Württemberg, BY: Bavaria, C: Central, E: East, M: Moravia, NE: North-East, Pyr: Pyrenees, RP: Rhineland-Palatinate, S: South, SL: Saarland, SW: South-West, TH: Thüringen, W: West; *Third part (only for western Germany):* Na: Nahe region, NE: northern Eifel, SE: southern Eifel, SM: Saar-Moselle region; *last part:* name of sampling locality

Hughes & Zalucki 1984; Zalucki *et al.* 1987; Rosenberg 1989; Goulson 1993; Johannesen *et al.* 1997). Therefore, we assume that western and eastern populations belong to two well distinguished lineages. We provisionally would regard these as subspecies or even subspecies complexes.

Reviewing the nomenclature of *P. coridon*, the presented data is not sufficient for a general taxonomic revision because no sample from the type locality, Graz, or its surroundings was included. Even the closest sample site, Rezi in western Hungary which belongs to the eastern genetic lineage, is located 150 east of Graz. Therefore, it is unceartain which group includes the nominate form.

The individuals from Brandenburg and other regions of East Europe and the north-eastern part of Central Europe are often somewhat larger and darker than more southern populations and therefore were separated for morphological reasons as *P. coridon borussia* (Dadd, 1908). However, we could not detect genetic differentiation of samples from Brandenburg to the others of the eastern lineage that would justify their subspecific separation (Fig. 2). Genetic differentiation among populations within the Brandenburg region was relatively high, possibly due to the marginal position of Brandenburg, where relatively isolated populations may have been subjected to increased genetic drift.

Genetic variability within populations. The observed genetic diversity within populations of *P. coridon* was high even for butterflies (Graur 1985; Packer *et al.* 1998; Schmitt 1999).

Literature data about butterflies indicate that taxa with strongly fragmented and small populations mostly express low allozyme diversity (e.g. Britten *et al.* 1994, 1995; Debinski 1994; Marchi *et al.* 1996), compared to widespread and common species which typically have high values (e.g. Goulson 1993; Porter & Geiger 1995; Porter *et al.* 1995; Schmitt 1999). *Polyommatus coridon* was abundant at nearly all sampling sites, so effects of genetic drift may be limited, despite the limited spatial extent of the habitat available in several regions.

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